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URGENT!!

Our Ref: PA/KIST99346

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AUSTRIAN PATENT OFFICE
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Re: International Application No. PCT/KR99/00414
in the name of Korea Institute of Science and Technology et al.

Dear Sirs:

The applicants wish to make claim amendments under Article 34 for the above-identified application in order to clearly distinguish the present invention from the cited references in the International Search Reports. Enclosed please find a replacement of pages 35 to 44 currently in the application.

In the amendment:

- Claims 9, 13 to 16, 18, 22 to 24, 53 to 58, 63 to 68, 71 and 72 *unchanged*;
- Claims 1, 2, 5, 6, 10 to 12, 17, 19 to 21, 25, 52, 60 to 62, 69, 70, 75 and 76 *amended*;
- Claims 3, 4, 7, 8, 26 to 51, 59, 73, 74, 77 and 78 *cancelled*; and
- Claims 79 and 80 *added*.

By the amendment:

- 1) In Claim 1, the emulsion type (oil-in-water) and intended use was specified. The term "cationic surfactant" was changed to "cationic lipid transfection agent".
- 2) In Claim 2, the intended use was specified and the term "cationic surfactant" was changed to "cationic lipid transfection agent".
- 3) Claim 5 was amended in compliance with the amended claim 1.
- 4) Claim 6 was amended in compliance with the amended claim 2.
- 5) In Claim 10, the expression "of step a)" was deleted.
- 6) In Claim 11, 75 and 76, the term "cationic surfactant" was changed to "cationic lipid transfection agent".

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- 7) In Claims 12 and 20, the term "cationic surfactant" was changed to "cationic lipid transfection agent" and "dimethyldioctadecylammonium chloride" was excluded.
- 8) In Claims 17, 19, 21, 25, claim dependency was changed.
- 9) In Claims 52 and 62, claim dependency was changed and "pharmaceutical drugs" was excluded from the exemplary biologically active materials.
- 10) In Claims 60 and 69, the drug was restricted to the scope as exemplified in the cancelled claim 35.
- 11) In Claims 61 and 70, the kind of anticancer drug is specified.
- 12) Claims 79 and 80 directed to the complex containing cyclosporin A as immunosuppressant are newly added.

Please conduct international preliminary examination in view of the amended claim set.
Should you have any queries, please let us know.

Very truly yours,
PARK, KIM & PARTNER

JWP/ejc
Encls.-replacement pages 35 to 44

Jang Won Park

Claims;

1. An oil-in-water lipid emulsion for delivering biologically active material selected
5 from the group consisting of DNA, RNA, antisense nucleic acid, ribosome, polynucleotide and oligonucleotide, comprising: 2-30% of non-triglyceride oil; 0.01-20% of one or more cationic lipid transfection agent; and, water to 100%.
2. Solid-lipid nanoparticles for delivering biologically active material selected from
10 the group consisting of DNA, RNA, antisense nucleic acid, ribosome, polynucleotide and oligonucleotide, comprising: 2-30% of fat of triglycerides having 10-18 carbons in each hydrophobic tail or ethyl stearate; 0.01-20% of one or more cationic lipid transfection agent; and, water to 100%.
- 15 3. Deleted
4. Deleted
5. A method of preparing an oil-in-water lipid emulsion for delivering biologically
active material selected from the group consisting of DNA, RNA, antisense
20 nucleic acid, ribosome, polynucleotide and oligonucleotide, comprising: a) a first step of preparing an aqueous phase by mixing 0.01-20% of one or more cationic lipid transfection agent with water and b) a second step of preparing emulsion of said aqueous phase with 2-30% of non-triglyceride oil.
6. A method of preparing solid lipid nanoparticles for delivering biologically active
25 material selected from the group consisting of DNA, RNA, antisense nucleic

acid, ribosome, polynucleotide and oligonucleotide, comprising: a) a first step of preparing an aqueous phase by mixing 0.01-20% of one or more cationic lipid transfection agent with water and b) a second step of mixing said aqueous phase with 2-30% of fat of triglycerides havin 10-18 carbons in each hydrophobic tail or ethyl stearate.

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9. The emulsion according to claim 1, further comprisign 0.01-10% of a hydrophilic polymer or hydrophilic polymeric lipid.

10. The emulsion accordign to claim 1, wherein the non-triglycerides is squalene or squalane.

11. The emulsion according to any of claims 1, 9 or 10, further comprising a phospholipid or a non-ionic surfactant.

12. The emulsion according to claim 1, wherein the cationic lipid transfection agent is selected from the group consisting of:

1,2-dimyristoyl-3-trimethylammonium-propane,

1,2-dipalmitoyl-3-trimethylammonium-propane,

1,2-distearoyl-3-trimethylammonium-propane,

1,2-dioleoyl-3-trimethylammonium-propane,

1,2-dimyristoyl-3-dimethylammonium-propane,

1,2-dipalmitoyl-3-dimethylammonium-propane,

1,2-dilauroyl-3-dimethylammonium-propane,

1,2-distearoyl-3-dimethylammonium-propane,

1,2-dipalmitoyl-3-trimethylammonium-propane,

5 N-[1-(1,2-dioleoyloxy)propyl]-N,N,N-trimethylammonium chloride,

1,2-dioleoyl-3-ethylphosphocholine, and other cationic lipids.

13. The emulsion according to any of claims 1, 9, or 10, further comprising glycerol or fusogenic peptides.

14. The emulsion according to claim 13, wherein the fusogenic peptide is
10 polyethylene glycol of MW.500-1000 or HA gp 41.

15. The emulsion according to claim 9, wherein the hydrophilic polymer is selected from the group consisting of polyoxyethylene, polyethyloxazoline and polyethyleneglycol.

16. The emulsion according to claim 11, wherein the phospholipid is selected
15 from the group consisting of phosphatidylcholin, phosphatidylethanolamine, phosphatidylserine, diacetylenic phospholipid and derivative thereof and the non-ionic surface active agent is selected from the group consisting of poloxamer, sorbitan ester, polyoxyethylene-sorbitan fat acid ester and polyoxyethylene ethers.

20 17. The emulsion according to any of claims, 1, 9, or 10, further comprising 1,2-dioleoyl-sn-3-phosphatidylethanolamine, diolein, fatty alcohol, cholesterol

or bile salt.

18. The solid lipid nanoparticles according to claim 2, further comprising 0.01-10% of a hydrophilic polymer or hydrophilic polymeric lipid.

19. The solid lipid nanoparticles according to claim 2 or 18, further comprising a phospholipid or a non-ionic surfactant.

20. The solid lipid nanoparticle according to claim 2, wherein the cationic lipid transfection agent is selected from the group consisting of:

1,2-dimyristoyl-3-trimethylammonium-propane,

1,2-dipalmitoyl-3-trimethylammonium-propane,

10 1,2-distearoyl-3-trimethylammonium-propane,

1,2-dioleoyl-3-trimethylammonium-propane,

1,2-dimyristoyl-3-dimethylammonium-propane,

1,2-dipalmitoyl-3-dimethylammonium-propane,

1,2-dilauroyl-3-dimethylammonium-propane,

15 1,2-distearoyl-3-dimethylammonium-propane,

1,2-dipalmitoyl-3-trimethylammonium-propane,

N-[1-(1,2-dioleoyloxy)propyl]-N,N,N-trimethylammonium chloride,

1,2-dioleoyl-3-ethylphosphocholine, and other cationic lipids.

21. The solid lipid nanoparticles according to claim 2 or 18, further

comprising glycerol or fusogenic peptides.

22. The solid lipid nanoparticles according to claim 21, wherein the fusogenic peptide is polyethylene glycol of MW.500-1000 or HA gp 41.

23. The solid lipid nanoparticles according to claim 18, wherein the hydrophilic polymer is selected from the group consisting of polyoxyethylene, polyethyloxazoline and polyethyleneglycol.

24. The solid lipid nanoparticles accordign to claim 19, wherein the phospholipid is selected from the group consisting of phosphatidylcholin, phosphatidylethanolamine, phosphatidylserine, diacetylenic phospholipid and derivative thereof and the non-ionic surface active agent is selected from the group consisting of poloxamer, sorbitan ester, polyoxyethylene-sorbitan fat acid ester and polyoxyethylene ethers.

25. The solid lipid nanoparticles according to claim 2 or 18, further comprising 1,2-dioleoyl-sn-3-phosphatidylethanolamine, diolein, fatty alcohol, cholesterol or bild salt.

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52. A complex of the emulsion according to any of claims 1, 9 to 17 and a biologically active material selected from the group consisting of DNA, RNA, antisense nucleic acid, ribosome, polynucleotide, oligonucleotide.
53. The complex according to claim 52, further comprising glycolipid, lipopeptide, antibody, ligand for receptors or viral protein to target specific cells or organs.
54. The complex according to claim 52 or 53, further comprising protamine sulfate, histone or cationic polymer.
55. The complex according to claim 54, wherein cationic polymer is polylysine.
56. The complex according to claim 52, further comprising monovalent or multivalent salt.
57. The complex according to claim 53, wherein the cell is selected from the group consisting of white blood cells, fibroblasts, cancer cells, cells infected with virus, epithelial cells, endothelial cells, muscle cells, liver cells, endocrine cells, neural cells, dermal cells, germ cells, oocytes, sperms, hematopoietic cells, fetal cells, M cells, Langerhans islet cells, macrophages, plant cells, animal cells, and immortalized cell lines.
58. The complex according to claim 52, wherein the complex is transferred to cells via intravenous, intramuscular, intratracheal, intranasal, subcutaneous,

parenteral or topical administration or through direct administration to a specific organ.

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60. The complex according to claim 52, further comprising lipophilic or
5 amphiphilic drug in an oil phase, wherein the lipophilic or amphiphilic drug is
selected from the group consisting of antivirals, steroidal anti-inflammatory
drugs, non-steroidal anti-inflammatory drugs, antibiotics, antifungals, vitamins,
hormones, retinoic acid, prostaglandins, prostacyclins, anticancer drugs,
antimetabolitic drugs, miotics, cholinergics, adrenergic antagonists,
10 anticonvulsants, antianxiety agents, major tranquilizers, antidepressants,
anesthetics, analgesics, anabolic steroids, estrogens, progesterones,
glycosaminoglycans, polynucleotides, immunosuppressants and
immunostimulants.

61. The complex according to claim 60 wherein the anticancer drug is taxol,
15 paclitaxel or flurouracil.

62. A complex of the solid lipid nanoparticles according to any of claims 2, 12
to 19 with a biologically active material selected from the group consisting of
DNA, RNA, antisense nucleic acid, ribosome, polynucleotide and
oligonucleotide.

20 63. The complex according to claim 62, further comprising glycolipid,
lipopeptide, antibody, ligand for receptors or viral protein to target specific cells
or organs.

64. The complex according to claims 62 or 63, further comprising protamine

sulfate, histone or cationic polymer.

65. The complex according to claim 64, wherein the cationic polymer is polylysine.

66. The complex according to claim 62, further comprising monovalent or
5 multivalent salt.

67. The complex according to claim 63, wherein the cell is selected from the group consisting of white blood cells, fibroblasts, cancer cells, cells infected with virus, epithelial cells, endothelial cells, muscle cells, liver cells, endocrine cells, neural cells, dermal cells, germ cells, oocytes, sperms, hematopoietic
10 cells, fetal cells, M cells, Langerhans islet cells, macrophages, plant cells, animal cells, and immortalized cell lines.

68. The complex according to claim 62, wherein the complex is transferred to cells via intravenous, intramuscular, intratracheal, intranasal, subcutaneous, parenteral or topical administration or through direct administration to a specific
15 organ.

69. The complex according to any of claims 62 to 68, further comprising lipophilic or amphiphilic drug in the fat, wherein the lipophilic or amphiphilic drug is selected from the group consisting of antivirals, steroidal anti-inflammatory drugs, non-steroidal anti-inflammatory drugs, antibiotics, antifungals, vitamins,
20 hormones, retinoic acid, prostaglandins, prostacyclins, anticancer drugs, antimetabolite drugs, mitotics, cholinergics, adrenergic antagonists, anticonvulsants, antianxiety agents, major tranquilizers, antidepressants, anesthetics, analgesics, anabolic steroids, estrogens, progestones,

glycosaminoglycans, polynucleotides, immunosuppressants and immunostimulants.

70. The complex according to claim 69, wherein the anticancer drug is taxol, paclitaxel or fluorouracil.

5 71. The method according to claim 5, wherein the aqueous phase further comprises 0.01-10% of a hydrophilic polymer or hydrophilic polymeric lipid.

72. The method according to claim 6, wherein the aqueous phase further comprises 0.01-10% of a hydrophilic polymer or hydrophilic polymeric lipid.

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75. The method according to claim 5, wherein the cationic lipid transfection agent is added in the oil phase instead of in an aqueous phase.

76. The method according to claim 6, wherein the cationic lipid transfection agent is added in melted fat instead of in an aqueous phase.

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79. The complex according to claim 60, wherein the immunosuppressant is cyclosporin A.

80. The complex according to claim 69, wherein the immunosuppressant is
20 cyclosporin A.